

## **REMARKS/ARGUMENTS**

### ***I. Status of the claims***

Claims 15, 23, 25, 31, 38 and 39 are amended. Claims 15-17, 23-26, and 31-41 are pending with entry of this Amendment.

### ***II. Support for the Amendments***

Support for the amendments can be found in the specification and claims as originally filed. Support for determining a non-linear continuously differentiable function of a logarithm of the initial copy number of target nucleic acid in the dilutions can be found in the specification, e.g., in the third through fifth paragraph of page 25 of the application. No new matter is added.

### ***III. Obviousness Rejection***

The Examiner rejected claims 15-17, 23-26, and 31-41 under 35 U.S.C. § 103 as allegedly obvious in view of Lowe *et al.* in view of Wittwer *et al.* The Examiner essentially repeated the rejection from the previous office action. The Examiner further argued that the claims do not recite determining initial copy number of each dilution and thus Applicants allegedly argued limitations that were not in the claims. *See*, Office Action, pages 5 and 7. The Examiner argued that the fluorescence measured in Wittwer *et al.* "is considered to determine the threshold value and which is correlated as a non-linear function in terms of a polynomial fit as disclosed by Wittwer *et al.*" *See*, Office Action, page 6. The Examiner concluded in stating: "Therefore the Wittwer *et al.* does teach determining a non-linear function and it is obvious to combine the method of Lowe *et al.* with the teachings of Wittwer *et al.* to achieve the instant invention." *See*, Office Action, page 7. Applicants respectfully traverse the rejection.

To set forth a *prima facie* obviousness rejection, the combination of references must describe all of the claimed elements. In the present rejection, neither cited reference do not include a description of determining a non-linear continuously differentiable function of a

logarithm of copy number as a function of the cycle number at which the signal threshold value is exceeded, as recited, for example in step (e) of claim 15.

The present claims currently involve steps that include making a dilution series of target nucleic acids (e.g., step (a) of claim 15), amplifying the nucleic acids in the dilutions (step (b)), and determining a non-linear continuously differentiable function of a logarithm of copy number as a function of the cycle number at which the signal threshold value is exceeded (step (e)). The determination of a non-linear function involves comparing the logarithm of the initial number of copies *in each dilution* (e.g., from step (a)) versus the cycle threshold. Applicants strongly dispute that the claims did not include this limitation previously as the relevant language in the determining step (for example of claim 15) have antecedent basis to earlier steps that refer to *each dilution* (e.g., steps (a) and (d) in claim 15) and because determining a function necessarily involves looking at multiple points (i.e., from multiple dilutions) to generate a non-linear function. Nevertheless, such language is now expressly inserted into each independent claim to remove any question the Examiner may have on this point. Again, neither reference cited describes determining, generating, or using a non-linear function as recited in the claims and therefore the cited references cannot and do not render the claims obvious.

To further support this point, Applicants submit a Declaration under 37 C.F.R. § 1.132 of Dr. Carl Wittwer, Ph.D., M.D. Dr. Wittwer is a named inventor of the Witter *et al.* patent cited by the Examiner. As detailed in the declaration, Dr. Wittwer confirms that the references do not teach or suggest a step of determining a non-linear continuously differentiable function of logarithm of initial copy number as a function of cycle threshold.

As explained in paragraph 5 of the Declaration, the Examiner correctly states that the '670 patent describes a method of DNA monitoring at each PCR cycle by measuring melting curves and calculating copy number at each cycle. However, the claimed invention involves determining a non-linear function of the logarithm of the initial concentration of nucleic acid in multiple dilutions and the cycle threshold. Monitoring amplification at each cycle, as described in the cited sections of the '670 patent, does not render it obvious to determine a non-linear function of the logarithm of initial copy number and cycle threshold.

As explained in paragraph 6 of the Declaration, the sections of the '670 patent cited by the Examiner (col. 3, lines 30-61; col. 4, line 45-63; col. 7, line 14-31; Figs. 22-23; and col. 17, lines 34-39) describe monitoring of amplification in real time (i.e., at every amplification cycle), but these sections do not teach or suggest the claimed non-linear relationship of the logarithm of initial copy number and cycle threshold. For example, the Examiner has cited col. 4, lines 45-63 of the '670 patent, which refers to a "3-dimensional spiral," for a teaching of "nonlinear functionality." *See*, December 23, 2005 Office Action, page 4, third paragraph. While the '670 patent does indeed refer to a "3-dimensional spiral," the spiral has nothing to do with the invention as currently claimed in the '712 application. Rather than teaching anything about the relation of the logarithm of initial copy number and cycle threshold, the "3-dimensional spiral" refers to measurement of temperature, time and fluorescence during each cycle of an amplification. *See*, the '670 patent, col. 4, lines 52-63. Measurement of temperature, time and fluorescence *within* an amplification does not suggest the relation of the logarithm of initial copy number and cycle threshold because these measurements do not involve analysis of dilutions of a target nucleic acid. Indeed, the use of the term "3-dimensional" does not relate at all to a mathematical function as recited in the claims of the '712 application. Therefore the Examiner is not correct in stating that the "3-dimensional spiral" discussed in the '670 patent has anything to do with the non-linear functions recited in the claims of the '712 application.

Figures 22 and 23 of the '670 patent, also cited in the Examiner's rejection, do not teach or suggest determining a non-linear function of the logarithm of initial copy number and cycle threshold. Figure 22 displays real time fluorescence of an amplification of one sample. Fluorescence information from one amplification does not provide information regarding the cycle threshold values from *different* dilutions that one of skill in the art would use to determine a function between the logarithm of copy number and cycle threshold. *See*, Declaration, paragraph 7.

Figure 23 displays real time fluorescence in relation to cycle number for different dilutions of a target nucleic acid. However, determining a non-linear function of the logarithm of initial copy number and cycle threshold is not suggested in Figure 23. Moreover, it was

commonly assumed before the filing of the '712 application that a *linear* function of the logarithm of initial copy number to cycle threshold should be determined. This is illustrated, for example, in the Lowe *et al.* reference (page 5, lines 7-8 and Figure 1B), each of which use *linear* regression to determine a *linear* function between the logarithm of initial copy number and cycle threshold, and in Figures 24, 26, and 28 of the '670 patent, which suggests good fit to a linear function. Nothing in the art the Examiner has cited contradicts this common assumption, i.e., that one of skill in the art should generate a *linear* function of the logarithm of initial copy number and cycle threshold. *See*, Declaration, paragraph 8.

In short, neither reference cited by the Examiner describes determining, generating, or using a non-linear function as recited in the claims. Moreover, to the extent the cited art describes any related process, the references lead those of skill in the art to generate a *linear* function. Finally, an inventor of one of the references cited by the Examiner have confirmed that the combination of references do not teach or suggest a step of determining a non-linear continuously differentiable function of logarithm of initial copy number as a function of cycle threshold as recited in the claims. *See*, paragraph 10 of the Declaration of Dr. Carl Wittwer, Ph.D., M.D. In view of these many reasons that the claims are not obvious, Applicants respectfully request withdrawal of the rejection.

Appl. No. 09/823,712  
Amdt. dated April 21, 2006  
Amendment under 37 CFR 1.116 Expedited Procedure  
Examining Group 1637

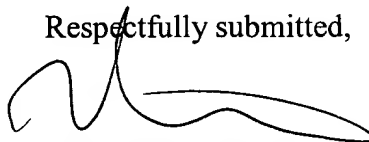
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**CONCLUSION**

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Matthew E. Hinsch', with a stylized, flowing script.

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